# ON THE REPRODUCTION AND LARVAL DEVELOPMENT OF STREBLOSPIO BENEDICTI WEBSTER [1,2]

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*Streblospio benedicti* Webster is a common but inconspicuous polychaete which abounds in many habitats along the eastern United States seaboard from New England to North Carolina. It is often present in estuaries, on beaches, or where the substratum consists of fine mud with considerable debris (Hartman, 1945).

This worm is very unusual for it is larviparous. The only published accounts of the reproduction or larval development of this species are brief comments by Campbell (1957) and Jones (1961).

The investigations reported here include descriptions of the development of larval specimens reared from plankton isolates and of larvae released by adults maintained in the laboratory.

## Methods

During June and July of 1963, *Streblospio* adults were collected from the Pequotsepos section of the Mystic River Estuary, where an abundant population inhabited a substratum of fine mud. In the laboratory the worms were maintained in fingerbowls containing sediment. The sediment came from the same area as the worms but was screened free of macrofauna, washed in distilled water, and air-dried prior to use.

Pelagic larvae were obtained from two sources: (a) isolates of qualitative plankton tows taken in the Mystic River Estuary with a No. 10 net during June to October, 1962 and 1963; (b) broods released by adults maintained in the laboratory. Larvae were reared in funnels, with and without sediment, as described previously (Dean and Hatfield, 1963a).

To maintain temperature control, both funnels and fingerbowls were partially immersed in a bath of flowing water from the laboratory's salt water system. Water in all containers was changed three times a week. Sea water used for cultures was Millipore-filtered (pads of 47 $\mu$ porosity) and stored in stoppered flasks in a bath of running sea water. Both adults and larvae were fed a suspension of liver powder weekly (Howie, 1958). Larvae and adults were maintained easily under these conditions in the laboratory. Adults formed new tubes rapidly in the sediment.

For examination, larvae were placed on microscope slides with small pieces of Saran Wrap used as coverslips (Dean and Hatfield, 1963a, 1963b). This was

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effective in quieting larvae without injury. Photographs, taken through a phase microscope, were obtained with a Polaroid camera using ten-second film. A series of photographs, descriptions and sketches of characteristic parts served as the basis for the composite outline drawings.

## I. Development Within the Adult

Worms were examined periodically for the presence of eggs.

Females could be distinguished from males only if the worms contained eggs or larvae. The blue-green eggs and larvae can be seen through the body wall. Uncleaved eggs measure 53 to 76 $\mu$ in diameter. The sperm of males imparts a faint milky appearance to the posterior region of the worm. This coloration is so slight that it was not observed until several dozen worms had been examined. Sperm length ranges from 100 to 130 $\mu$. An unusually long midpiece (*ca.* 60 $\mu$) is found in these sperm.

Thirty gravid females, ranging from 40 to 50 segments in length, were isolated in stender dishes containing a small amount of sediment. These worms were examined frequently in order to study the development of young.

All larval development was restricted to the middle portion of the worms. The first egg-containing segment ranged from the 11th to the 16th (average 12.8). The 31st segment was the last to contain larvae. The posterior 6 to 10 segments of this reproductive zone developed paired dorso-lateral swellings—the brood pouches—from which the matured larvae were released. One pair of eggs or larvae usually occupied a segment, although one pair of larvae per pouch was observed in a few instances.

The young developed in cycles, with several maturing at about the same time. The release of larvae began at the most anterior pouch and progressed segment by segment. Normally it took two to three hours for all larvae in the pouches to be liberated. Two to three days following the release of a larval brood, another set of larvae occupied the brood pouches. These were in turn released about four to five days later. Refilling the brood pouches was sometimes associated with the disappearance of eggs from the anterior segments. However, neither eggs nor larvae were observed moving from one segment to another.

Forward of the brood pouches, development progresses in an anterior to posterior direction. Uncleaved or cleaved eggs occur anteriorly while trochophores slowly revolve in the segments near the brood pouches. When the brood pouches are refilled, the specimens occupying the posterior pouches are further advanced in development than those in the anterior pouches. Since all larvae are approximately equal in development at the time of release, either the larvae in the posterior pouches have a retarded rate of development or those in the anterior pouches have an accelerated rate.

Specimens ranging from uncleaved eggs to well-formed setigerous larvae were removed from females and examined under the phase microscope. Cleavage is spiral and leads to a trochophore (Fig. 1). The latter has a complete prototroch, one pair of eyes, sensory bristles on the head, and a neurotroch leading from the mouth. No apical tuft was observed. As development proceeds, a second pair of eyes is added, and the first segment—which is as broad as the head—becomes delimited. A distinct groove (Fig. 2) appears between the head and first segment, only to disappear later as the first segment becomes smaller than the head. A narrow, non-segmented body region extends posteriorly from segment 1. At this stage, although setae are absent, the total length approximates that of a 7-setiger larva. A mid-dorsal separation of the prototroch occurs, a telotroch develops, and within a period of about 24 hours, rapid setation and segmentation result in the formation of a 7-setiger larva (Fig. 3). As this takes place, the segments become delimited in succession from anterior to posterior, with the swimming setae being formed prior to the boundaries of a segment. No distinction can be made between noto- and neurosetae at this time, except a pair of hooded crotchets developing internally within the 7th setiger.

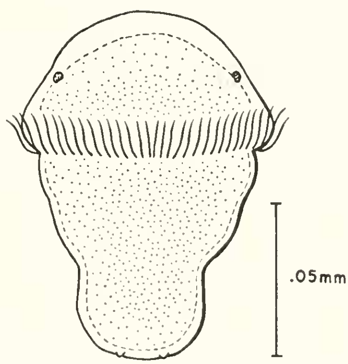


FIGURE 1. Early trochophore in dorsal view. Specimens of this stage are usually located in the female anterior of the brood pouches. Figures 2, 4 and 7 are drawn to the same scale.

All future segments are formed from a region just anterior to the pygidium. Formation of the 8th and 9th setigers takes about one day each. As setiger 9 develops, noto- and neurosetae become distinct. All notosetae are capillary, as are the neurosetae on setigers 1 through 6. Posterior to setiger 6, hooded crotchets develop and are accompanied by a stout, short, curved, capillary-tipped seta, one per neuropodium.

Larvae up to the 9-setiger stage were observed within females. Once in the brood pouches, the larvae may be released if the female is stimulated. In the laboratory, rough handling or subjection to strong light (for example, the light of a dissecting microscope lamp) may evoke the release of larvae from brood pouches. With careful handling of females, larvae were not released prior to the 9-setiger stage.

## II. Development of Planktonic Larvae

### 1. *General*

Although thousands of *S. benedicti* larvae were observed in plankton samples taken during two summers, none were found younger than 7-setiger or older than 13-setiger stages. Seven-setiger larvae were extremely rare; the vast majority of planktonic larvae were 9- and 10-setiger; 11-setiger stages were found less frequently; and 12- and 13-setiger stages were rarely encountered. The robust anterior end and the lack of chromatophores are the most obvious features that distinguish larvae of this species from all other planktonic larvae occurring in this area. In the youngest planktonic larvae the head region is approximately twice the width of the segmented portion. In later stages this characteristic becomes less marked although there is still an illusion of greater mass in the anterior region caused by the development of palps, branchiae, and the collar of setiger 2.

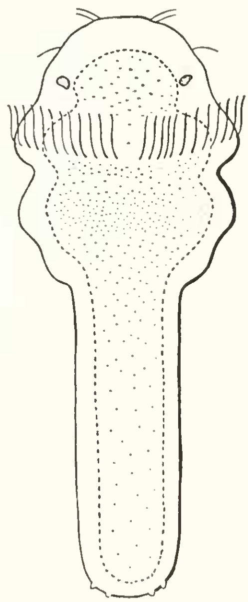

FIGURE 2. Late trochophore liberated from a brood pouch following stimulation of a female. Dorsal view.

*2. Detailed description*

All pelagic stages have four red eyes in a transverse row and neuropodial hooded tridentate crotchets beginning on setiger 7 (Figs. 3 to 7). In the 7- to 9-setiger stages, the neuropodia have a single crotchet accompanied by a short, curved, capillary seta. A second crotchet is added at the 10- or 11-setiger stage. The first six neuropodia and all notopodia have capillary setae only. When new segments are added, the crotchets are the first setae to appear. Swimming setae of early planktonic stages are very long; those of setiger 1 extend beyond the pygidium. These finely serrated setae break off at the slightest provocation and are of little diagnostic value. Eleven-setiger stages obtained from plankton tows usually lack swimming setae.

The prototroch persists through the 13-setiger stage. It encircles the swollen head region except in the dorsal midline and is outlined by a red-brown line at the base of the cilia. The prototroch is lost between the 13- and 14-setiger stage. Ventrolaterally the prototroch is borne on lobes (Fig. 4). The latter are fused anteriorly and bound the mouth laterally. A V-shaped neurotroch passes posteriorly from the triangular-shaped mouth to a ciliated pit on the anterior aspect of setiger 2 (Fig. 6).

The telotroch is present on 7- to 13-setiger stages. It is composed of 5 patches of cilia (Figs. 4, 6 and 7). The mid-ventral patch marks the anterior boundary of a ventral groove on the pygidium (Fig. 6). Only the lateral patches are seen in dorsal view (Fig. 5). The telotroch begins to disappear as the 14th setiger develops.

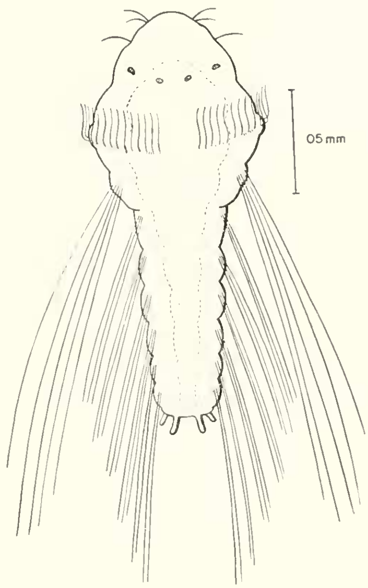


FIGURE 3. Dorsal view of a 7-setiger larva. Telotroch omitted. Figures 5 and 6 are drawn to the same scale.

Prominent gastrotrochs, arranged in four patches across setigers 4 through 9, are present in all planktonic stages (Figs. 4, 6 and 7). Thinner, shorter, inconspicuous cilia may be seen ventrally on segments 1, 2 and 3 of some larvae. Nototrochs are absent in pelagic larvae of this species. Four stiff sensory cilia are present on the prostomium of all pelagic larvae.

Palp buds arise in the 9-setiger stage as small dorsolateral outgrowths anterior to the prototroch (Fig. 5). Although triangular in shape initially, the palps elongate and become highly mobile by the 12-setiger stage.

Branchial *Anlagen* appear in the late 9- or early 10-setiger stage, dorsal to the notosetae on setiger 1. In the 11-setiger stage, branchiae are longer than the palps. However, by the time setiger 13 is added, the palps are approximately twice the length of the branchiae. Branchial ciliation and apical tufts of sensory cilia arise in the 10- to 11-setiger stage.

The collar on setiger 2 becomes apparent on the 11-setiger stage.

Pigmentation of pelagic larvae is restricted to a red-brown line at the base of the prototroch, a similarly colored area on the pygidium, and a pale blue-green region at the base of the swimming setae on setiger 1.

The size and number of anal cirri are variable (Figs. 1 to 7). Although four is the most frequently encountered number, the cirri vary from zero to four. There seems to be no relationship between size or number of anal cirri and the stage of development. Anal cirri begin to disappear during metamorphosis.

## III. Metamorphosis and the Length of Larval Life

Metamorphosis is an orderly sequence of events that is completed within a period of 24 hours to more than two weeks, depending upon the stage of larval development and the presence of a suitable substratum. Metamorphosis begins as the larva settles and crawls over and between the particles of sediment. Any

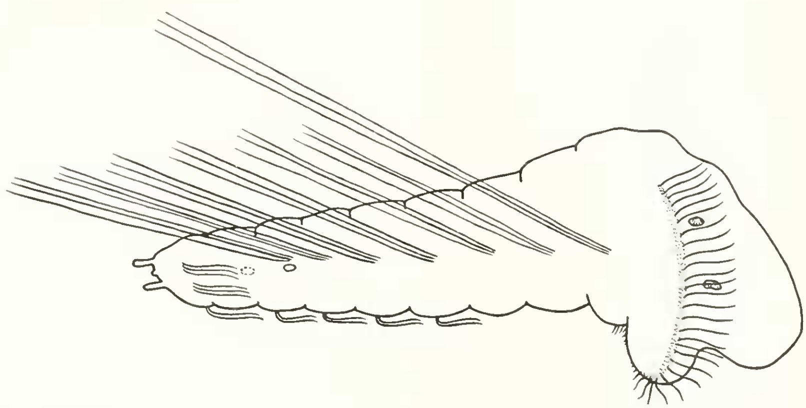

Figure 4. Lateral view of a 7-setiger larva. A crotchet can be seen developing internally in setiger 8. Sensory bristles omitted.

remaining swimming setae are lost during the process of the larva's crawling. A few sand grains adhere to the body wall and are carried along with the larva. As other grains are added, a stationary tube is formed within which the larva can move freely. Concomitant with crawling and tube formation is the gradual loss of the prototroch and then the telotroch. As these cilia disappear, the rate of development of branchiae and palps increases. Loss of the telotroch marks the end of the transition from free-swimming larva to benthic form.

An experiment to test the influence of sediment upon metamorphosis was performed as follows. On August 5, 1963, a female *S. benedicti* was stimulated and 15 larvae were released. These larvae were of 7-setiger size but had not completed the setation and segmentation of the 7-setiger stage. That is, they were at a stage between those shown in Figures 2 and 3. The larvae were divided into four funnels: two with sediment and two without sediment, as controls. On August 8, larvae in the funnels containing sediment had 9 well-developed setigers and had the setae of the 10th partially formed. Only the ventral cilia of the prototroch remained; some of the telotroch cilia had disappeared; and the palps and branchiae were well developed. Metamorphosis was near completion. Larvae in the control funnels were identical in setation to those in the funnels with sediment. However, both prototroch and telotroch were fully formed, and neither palps nor branchiae had begun to develop. By August 12, larvae in the controls had attained the 11-setiger stage and had well-developed palps and branchiae. The controls had developed no farther by August 19 and the experiment was terminated.

The youngest developmental stage that reached metamorphosis successfully was the late trochophore (Fig. 2). In this instance, a brood of 11 late trochophores was released by a stimulated female on August 12, 1963. Larvae from this brood

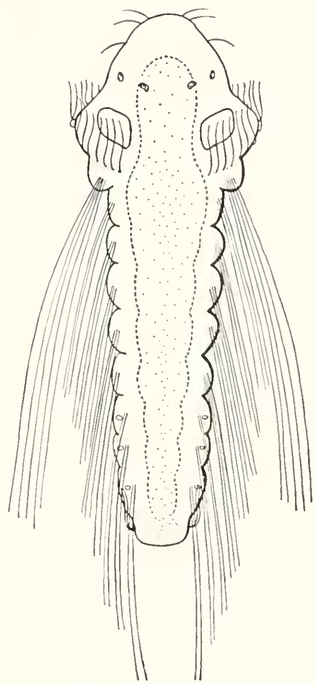

FIGURE 5. Dorsal view of a 9-setiger larva.

were divided into two groups: one with sediment and one without sediment. Those provided with sediment had reached the 10-setiger stage and had completed metamorphosis by August 19. Those maintained without sediment disintegrated prior to metamorphosis.

Metamorphosis accelerates the development of palps and branchiae. When, for example, 11-setiger stages from the same larval brood are compared, those having metamorphosed exhibit markedly larger palps and branchiae than unmetamorphosed larvae maintained in vessels without sediment.

All larvae, which were released by females in the laboratory and which were provided with sediment soon after liberation, began metamorphosis at the 9-setiger stage and had completed metamorphosis by the 10-setiger stage. Nine- to 13-setiger *S. benedicti* larvae isolated from plankton samples metamorphosed readily

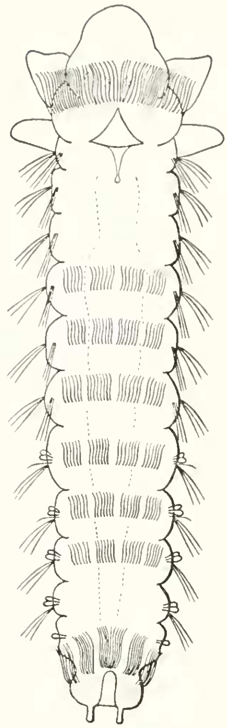

FIGURE 6. Ventral view of an 11-setiger larva. Sensory bristles and ciliation of palps, branchiae and oral region omitted.

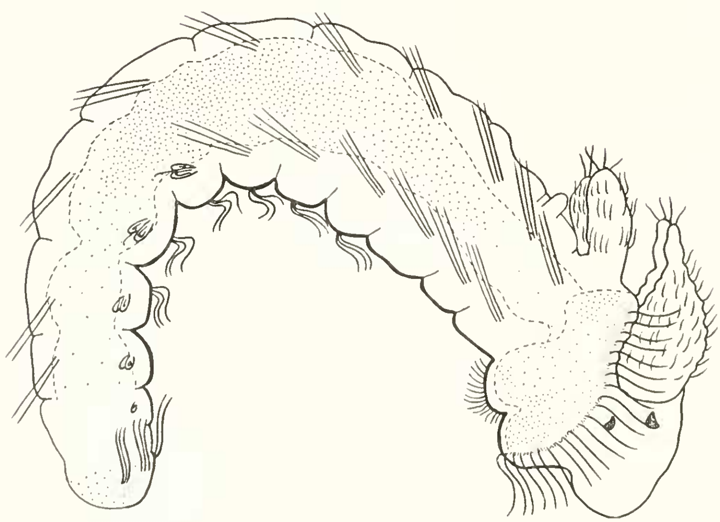

FIGURE 7. Lateral view of an 11-setiger larva. Sensory bristles omitted.

when they were placed in rearing vessels containing sediment. When similar isolates were maintained without sediment, the larvae would usually reach the 13-setiger stage before disintegrating. Only two specimens metamorphosed successfully in the absence of sediment. In both cases, metamorphosis coincided with the addition of setiger 14.

## Discussion

Both larvae at the time of hatching and eggs within females showed the characteristic blue-green coloration reported by Campbell (1957). Shortly after hatching, all coloration is lost except a small area at the bases of the swimming setae on setiger 1.

Several differences exist between the results of the present study and those reported by Campbell (1957). She noted that larvae, when released from the female, had three to four segments; had well developed prototrochs and telotrochs; and had serrated swimming setae, two pairs of red-brown eyes, and four anal cirri. In the present study, however, larvae first attained a length approximating that of a 7-setiger larva before a telotroch was formed or before setation or segmentation took place. It was also found that, unless disturbed, a female would retain larvae until the 9-setiger stage. These observations are supported by the size of *Streblospio* larvae found in the plankton of the Mystic River Estuary. Of the thousands of *Streblospio* larvae observed in plankton samples taken during two summers of study, none were found to have less than 7 setigers. The liberation of 3- and 4-segment stages reported by Campbell (1957) could be explained by rough handling of adults or by differences in reproductive behavior of this species in the two areas.

The spawning season of *Streblospio benedicti* in the Mystic River Estuary is from June to October. During this period the sexes can be distinguished if the females are ovigerous or if the pale, milky posterior region of ripe males is discernible. The sperm is of the aberrant type, a characteristic in keeping with its unusual mode of reproduction (Franzen, 1956).

## Summary

1. The larval development of the larviparous spionid polychaete, *Streblospio benedicti,* is described. Specimens were obtained from adults maintained in the laboratory and from plankton isolates. Early and late trochophores, 7-, 9- and 11-setiger stages are illustrated.

2. Undisturbed adult females retain larvae until the 9-setiger stage. If stimulated, however, the female will release younger stages. Under laboratory conditions, late trochophores and all later stages can be reared through metamorphosis. Larvae can metamorphose once they acquire 9 setigers, but they can delay metamorphosis until the 13-setiger stage in the absence of a suitable substratum. Rarely will larvae metamorphose in the absence of sediment. Pelagic life is usually three days or less but can be prolonged for at least two weeks.

3. Seven- to 13-setiger *S. benedicti* larvae occur in the plankton of the Mystic River Estuary from June to October. Nine- and 10-setiger larvae are encountered most frequently.

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